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The effect of fenugreek seed extract in the protection against gentamicin nephrotoxicity: a biochemical and histological study

Efficacy of fenugreek seed extract against gentamicin-induced nephrotoxicity

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Abstract

Aim: Gentamicin (GM) is an effective antibiotic against many Gram-negative bacterial infections, but it has a narrow therapeutic range. The application of gentamicin causes nephrotoxicity due to its accumulation in renal cortex and the oxidative stress it causes. The present study was aimed to carry out comparative biochemical and histopathological studies on the efficacy of niphedipine, fenugreek (FG) seeds against gentamicin-induced nephrotoxicity in rats.

Materials and Methods: Nephrotoxicity was induced in rats by administering gentamicin IP for 9 days at a dose of 125 mg/kg. The nephroprotective effect of the orally administered water extract of fenugreek seeds (FG) at a dose of 200 mg/kg or niphedipine (NF) at a dose of 20 mg/kg individually or in combination were studied. In order to determine the nephroprotective role of the compounds, serum biochemical parameters analyses and histology studies on kidney were performed.

Results: Reduction in weight of body, liver, right kidney and heart in rats treated with gentamicin was rescued with the help of fenugreek seed extract and nifedipine. Fenugreek and nifedipine treatment also reduces uric acid levels in the gentamicin treated group as compared to the group treated with gentamicin alone; no such change was observed in serum creatinine levels. Histological studies demonstrated the protective role of fenugreek and nifedipine against the extensive damage caused by the gentamicin treatment.

Discussion: In conclusion, this study showed potential or mild protection of fenugreek seed extract and nifedipine on gentamicin-induced nephrotoxicity in rats.

Keywords

Nephrotoxicity; Gentamicin; Fenugreek Seeds; Nifedipine

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Introduction

Gentamicin, an aminoglycoside antibiotic, is widely used to treat a variety of infections caused by Gram-negative (e.g. - ocular, pulmonary, and intestinal infections) or Gram-positive bacteria (e.g.-endocarditis) [1]. Treatment with gentamicin also results in unwanted toxic effects, nonetheless, it is still used as an effective therapeutic alternative against the bacteria that are resistant to other antibiotics. Widespread use of gentamicin can be primarily attributed to its chemical stability, fast bactericidal property, synergy with beta-lactam antibiotics, low resistance, and low cost of treatment [2]. However, the usefulness of this drug is limited due to its nephrotoxic and ototoxic effects [3]. Therefore, measurements of serum levels and dose regimen adjustment are important to avert toxicity.

The incidences of gentamicin nephrotoxicity have progressively increased since the time of its introduction despite accurate control and follow-up of the patients [4, 5]. According to a study, up to 30% of the patients who are treated with aminoglycosides for more than seven days, show signs of nephrotoxicity [6]. It has been reported that gentamicin nephrotoxicity is a consequence of the reduction in total antioxidant capacity of renal tissue and plasma coupled with increased synthesis of lipid peroxidation products [7].

Fenugreek seed extract (dose-400 mg/kg) was found to possess antioxidant activity and conferred protection against anticancer drug hepatotoxicity [8]. Furthermore, its antioxidant property protected testicles against doxorubicin-induced toxicity [9]. It has also been reported that fenugreek seed extract protects against pesticide-induced nephrotoxicity [10].

Previous studies have demonstrated that treatment of rats with nifedipine (10, 20 mg/kg) significantly reduces renal dysfunction, total nitric oxide levels in tissue and urine, renal oxidative stress, and also prevents alterations in renal morphology, all of which were caused by cyclosporine A exposure [11]. Nifedipine administered to rats at a dose of 15 mg/kg/day has been shown to confer protection against gentamicin nephrotoxicity [12].

Since oxidative stress plays an important role in gentamicin nephrotoxicity, antioxidant compounds such as fenugreek seed extract can help in significantly reduce the ill effects. Fenugreek seed extract also showed renoprotective ability against diabetic nephropathy.

This study was aimed to test the synergistic effects of fenugreek seed extract and nifedipine to alleviate gentamicin induced nephrotoxicity in male Wistar rats. The effectiveness of fenugreek seed extracts alone and a combination of fenugreek seed extract with nifedipine were assessed for the protection against gentamicin nephrotoxicity using both biochemical and histological studies.

Material and Methods

The study was approved by the Research ethics committee, Faculty of Medicine, King Abdulaziz University (KAU), Jeddah, Saudi Arabia.

Plant Material

Fenugreek seeds were purchased from the local market, cleaned of extraneous matter and were finally soaked in boiling distilled water (DW) (20 g/100ml) for 1h. The supernatant was used as an aqueous extract. The extract was prepared daily.

Chemicals

All chemicals were obtained from Sigma Chemical Co, USA. Gentamicin and nifedipine were purchased from Sigma Aldrich (St. Louis, MO, USA).

Study design

Healthy adult male albino Wistar rats (n= 30) weighing between 200- 250 g and aged 2-3 months, were used throughout this study. Standard conditions were maintained for the animals, allowing free access to rodent diet and water ad libitum. Animals were weighed before and after the experiment. Hygienic conditions were maintained for the animals who were individually housed in metabolic cages under 12 h light and dark cycles.

The rats were randomly divided into five groups (n=6) as follows:

Group 1: Served as a control. Kept on a normal diet and water on ad libitum .

Group 2: Injected intraperitoneally with gentamicin 125mg/kg for 9 days and served as animal model of gentamicin nephrotoxicity.

Group 3: Treated with nifedipine 20 mg/kg orally, in addition to gentamicin.

Group 4: Received water extract of fenugreek seed orally at a dose of 200 mg/kg twice daily for 9 days starting from the day of gentamicin injection.

Group 5: Animals were injected with gentamicin and received a combination therapy of nifedipine 20 mg/kg orally and fenugreek seed water extract for 9 days. All the groups were provided with food and water ad libitum.

After 9 days, rats from all the groups were anesthetized using diethyl ether.

Post the treatment period, animals were made to fast overnight. On a subsequent day, blood samples were collected from orbital sinus and stored in plain tubes. Serum was separated from the blood centrifuging the samples at 3500 rpm for 10 min. Serum was then divided into several aliquots and stored at -80°C until further analysis. Sera were further used to assay total antioxidant status, kidney functions tests (creatinine, urea, uric acid, and blood urea nitrogen (BUN) and albumin, using commercially available kits (Flex® reagent cartridge, Newark, USA).

Histological study:

First, animals were anesthetized and then were sacrificed by cervical dislocation. Kidneys were dissected out quickly, weighed, and finally fixed in 10% neutral buffered formalin. Right kidney was then processed for histological evaluations. Sections of 7.0 µm were cut from the tissues. Some sections were stained with Periodic Acid Schiff (PAS), and the rest with Hematoxylin and Eosin. For electron microscopy, small pieces of kidney including cortex and medulla were fixed in 3% glutaraldehyde in phosphate buffer (pH 7.4).

The scoring of glomerular histopathological changes and mesangial lesions was done by semi-quantitatively measuring the glomerular mesangial expansion (increase in the mesangial matrix). A four-point scale (normal; mild segmental mesangial expansion up to 50%; 2- mild diffuse expansion; 3- more than moderate diffuse expansion) was used to score mesangial expansion [9].

Preparation and staining of the sections were done according

to a previously described protocol [13]. Stained sections were examined under Olympus EX51 light microscope. Images of the sections were taken using the digital camera attached to the microscope and the computer with pro-image analyzer software.

Statistical Analysis

Statistical analyses were performed using SPSS software version 20. Data are presented as mean ± standard derivation (SD). Analyses between parameters were performed using the one-way ANOVA test for unrelated data. P-value of less than 0.05 was considered significant.

Results

During the initial experiments, body weights of the rats in different groups were measured. The body weights in Fenugreek and Gentamicin + Nifedipine groups were significantly higher than the control and Gentamicin treated groups (P =0.0001 for all); meanwhile, the body weight observed in the Gentamicin + Fenugreek + Nifedipine group was significantly lower than the control group (P =0.016) (Table 1). During further investigations, liver weight in the fenugreek treated group was found to be significantly higher (P =0.001) as compared with the control whereas, liver weight observed in the Gentamicin group was found to be significantly lower than all other groups (Table 1). Right kidney weight in Gentamicin + Nifedipine group was significantly higher than the control and gentamicin treated groups (P =0.01 and P =0.022) (Table 1). Left kidney weight was found to be significantly higher in control group than the Gentamicin, Fenugreek, Gentamicin + Fenugreek, Gentamicin + Nifedipine and Gentamicin + Fenugreek + Nifedipine groups (P =0.007, P =0.007, P =0.014, P =0.0001 and P =0.008, respectively); meanwhile, left kidney weight of Gentamicin + Nifedipine group was significantly higher than the gentamicin treated group (P =0.010) (Table 1). Next, heart weight in different groups was measured and it was found that heart weight in Fenugreek and Gentamicin + Nifedipine groups was significantly higher than the gentamicin treated group (P =0.002 and P =0.048, respectively) (Table 1). Further, left testis weight in different groups was measured, and it was observed that testis weight in the Gentamicin + Fenugreek + Nifedipine group was significantly lower than the control group (P =0.021) (Table 1).

Further, the percentage of liver weight in the Gentamicin + Fenugreek + Nifedipine group was found to be significantly higher than the gentamicin treated group (P =0.020) (Table 2). Similarly, the percentage of right kidney weight in the Fenugreek group was significantly lower than gentamicin treated group (P =0.011) (Table 2). In control and Fenugreek groups, the percentage of left kidney weight was significantly lower than the gentamicin treated group (P =0.008 and P =0.002) (Table 2). Further, in different groups, comparison of kidney and liver function tests and lipid profile was done. Serum creatinine level in Gentamicin + Fenugreek and Gentamicin + Nifedipine groups was significantly higher than control (P =0.014 and P =0.045), meanwhile serum creatinine level in control and Fenugreek groups was significantly lower than the gentamicin treated group (P =0.02 and P =0.021) (Table 3). Upon analysis of the uric acid levels, Gentamicin + Fenugreek, Gentamicin + Nifedipine and Gentamicin + Fenugreek + Nifedipine groups showed significantly lower uric acid level as compared to the gentamicin treated groups (P =0.005, P =0.009 and P =0.019, respectively) (Table 3). Serum cholesterol level in the Gentamicin + Fenugreek and Gentamicin + Nifedipine groups was significantly higher than the control (P =0.006 and P =0.0001); meanwhile, serum cholesterol level in the Gentamicin + Nifedipine group was found to be significantly higher than the cholesterol levels observed in gentamicin treated group (P =0.005) (Table 3). Triglyceride serum level in Gentamicin and Gentamicin + Fenugreek groups was significantly higher than control group (P =0.005 and P =0.0001), on the other hand, triglyceride serum level in Gentamicin + Fenugreek group was significantly higher than gentamicin treated group (P =0.025) (Table 3). Low-density lipoprotein cholesterol in Gentamicin + Nifedipine group was significantly higher than control and Gentamicin treated groups (P =0.0001 for both) (Table 3). Similarly, high-density lipoprotein cholesterol level in the Gentamicin + Nifedipine group was significantly higher than control and Gentamicin treated groups (P =0.0001 and P =0.009) (Table 3).

Histology of control rat kidney

The histological structure of the kidney in the control group was similar to that reported in previous studies. The cortex contains renal corpuscles with its two components. Bowman capsule lined by simple squamous epithelium, glomerular capillary tuft is present with normal cellular density, renal tubules showing intact epithelium, and normal lumina, free of any cell debris or casts (Figure 2). Similar results were observed in the proximal tubule sections, under electron microscope, where normal epithelial cells were observed with euchromatic rounded nucleus, intact brush border and normal population of mitochondria (Figure 5).

Histology of kidney in gentamicin administrated group

In this group, features of induced nephrotoxicity were observed. Marked damage of renal tubules was present in the form of atrophy or damage of lining epithelium, dilatation of their lumina, and the presence of cell debris and casts (Figure 1). Atrophy and lobulation of glomerular tufts were observed, and infiltration of interstitial inflammatory cells was evident in some samples (Figure 1). Electron microscopic assessment showed accumulation of lipids within basal parts of the epithelial cells, deformed mitochondria with vesiculated cristae and smaller nucleus (Figure 5).

Histology of kidney in gentamicin + fenugreek seeds administrated group

In this group, variation was observed in response to fenugreek protection. Moderate to higher protection was observed where the number of affected glomeruli and damaged tubules decreased, but in other samples, no improvement or even exaggerated damage was observed (Figure 2). The electron microscopic evaluation showed potential protection where proximal tubule cells showed normal nucleus, brush border clumped, few mitochondria and cells containing irregular dense body, most probably degenerated organelles near lysosomes (Figure 5). The rest of cytoplasm was depleted from cell organelles (Figure 5).

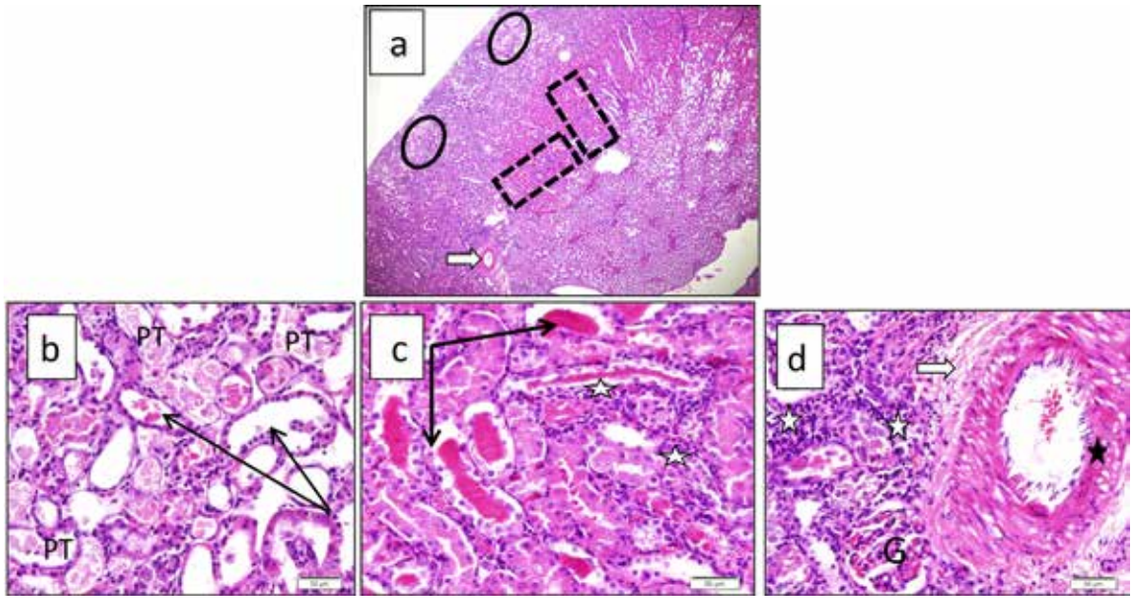

Figure 1. Sections of rat kidney of gentamicin treated group (G2). (a) Low magnification image of the regions of distal tubules with casts (dotted squares). Degenerated proximal tubules and altered arcuate artery are shown (circles). (b) High magnification image of tubules showing proximal tubule (PT), which is surrounded by lining cells with the presence of luminal casts. Distal tubules showed dilation due to atrophy of lining cells (arrows). (c) Shows highly acidophil proteinous casts within distal tube lumina (black arrows), and interstitial cell infiltrate (stars) (d) Thickened arcuate artery with perivascular fibrous deposition, vacuolation of media smooth muscles (black stars). Interstitial inflammatory cell infiltration can be observed (white stars). Glomeruli (G) showed decreased cellularity and lobular segmentation.

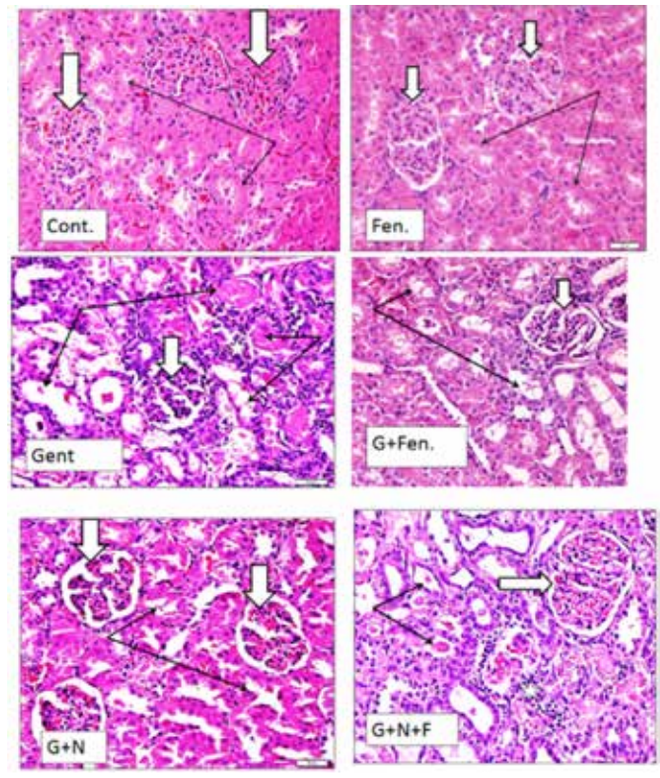

Figure 2. Sections from rat kidney cortex. Cont: Represents control with normal renal corpuscles and glomeruli (white arrows) and intact renal tubules (black arrows). Fen: Fenugreek group shows more healthy glomeruli (white arrows) and renal tubules (black arrows). Gent: Gentamicin group shows marked damage to tubular epithelium, dilatation of their lumina and presence of casts (black arrows), atrophy and lobulation of glomeruli (white arrow) and interstitial inflammatory cells (star). G+ Fen: Gentamicin + fenugreek seeds treated group showing potential or mild protection against gentamicin-induced nephrotoxicity. G+N: Gentamicin + Nifedipine group showing moderate protection against gentamicin-induced glomerular (white arrow) and tubule damage (black arrows). G+N+F: Gentamicin administered in combination of fenugreek seeds and nifedipine shows protection of glomeruli (white arrow), but no protection in case of renal tubule damage (black arrows), also highlighted by the presence of interstitial inflammatory cells (star).

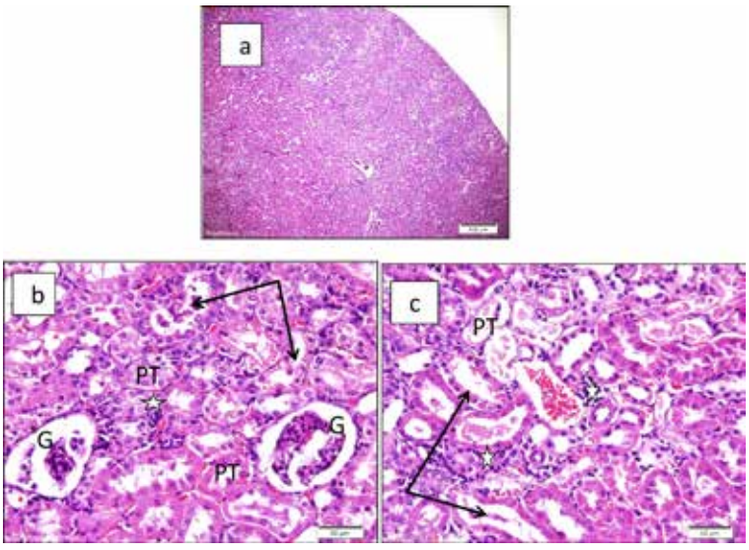

Figure 3. (a-c): Sections from gentamicin group treated with nifedipine shows less damaging effect on distal tubules (black arrows), decreased frequency of infiltrating interstitial inflammatory cells (stars). Few proximal tubules (PT) also showed degenerative changes or slight changes.lobular segmentation.

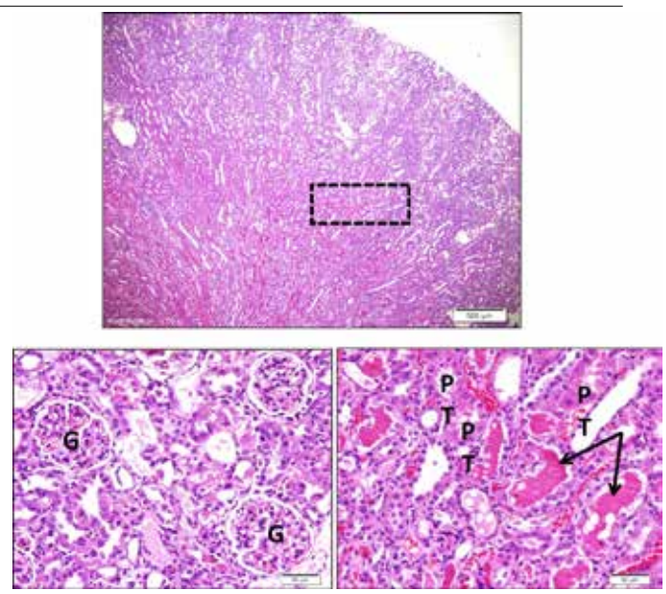

Figure 4. Sections from gentamicin group treated with both fenugreek and nifepedipine shows protection of only proximal tubules (PT) and glomerular component (G). Distal tubules still show dilation, epithelial atrophy and luminal casts (arrows).

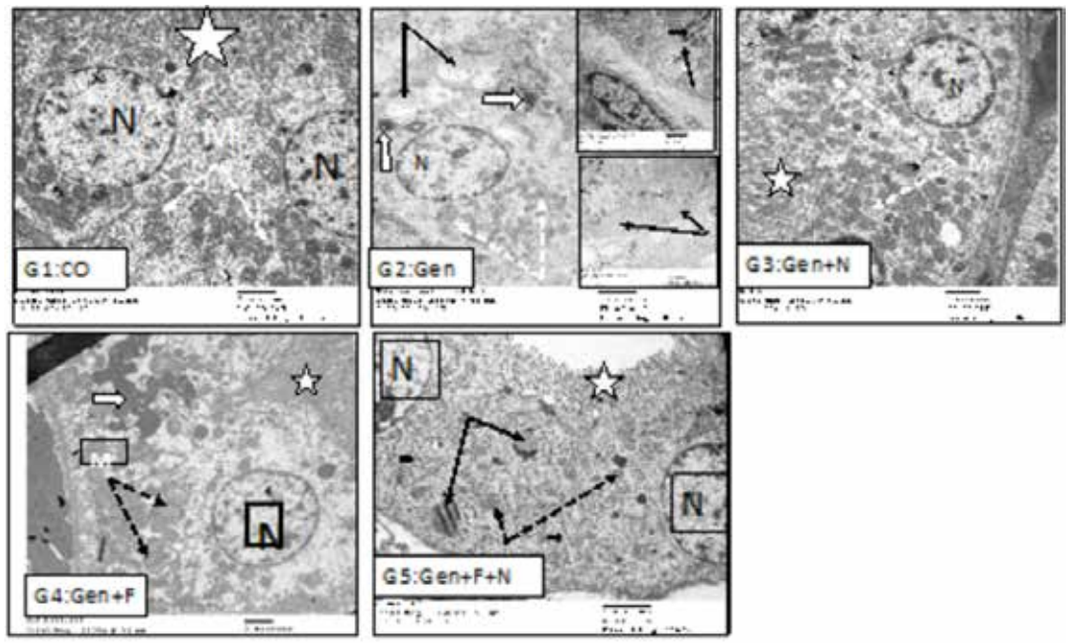

Figure 5. Electron micrograph of the sections of proximal tubules epithelium.G1: CO - Group 1 (Control) showing normal epithelial cell with euchromatic rounded nucleus (N), intact brush border (white star) and normal population of mitochondria (M. dotted arrows). G2: Gen - Group 2 (Gentamicin) showing accumulation of lipids within basal parts of the epithelial cells (black arrows), deformed mitochondria with vesiculated cristae (M. dotted arrows). Nucleus looks smaller (N). The insert shows similar changes. G3: Gen+N - Group 3 (Gentamicin + Nifedipine) showing proximal epithelial cells with normal brush border (star), mitochondrial population (M. dotted arrows) and nucleus (N). G4: Gen+F - Group 4 (Gentamicin + Fenugreek) demonstrates potential protection where proximal tubule cells showed normal nucleus (N), brush border clumped (white star), few mitochondria (dotted arrows), and cells contain irregular dense body, most probably degenerated organelles near lysosomes (white arrow). The rest of the cytoplasm is depleted from cell organelles (black star). G2: Gen+F+N - Group 5 (Gentamicin + Fenugreek + Nifedipine) shows intact cells with normal nuclei (N) and healthy brush border (star). The cells contain lipid droplets (black arrows). Also, mitochondria are few and deformed (white dotted arrows).rows).

Table 1. Comparison of changes in body and organs weights in different studied groups.

Parameters	Control untreated	Gentamicin treated	Fenugreek	Gentamicin + Fenugreek	Gentamicin + Nifedipine	Gentamicin + Fenugreek + Nifedipine
Body weight (gram)	223.48±12.79	215.33±4.84	296.98±0.91	227.48±10.72	274.65±2.91	201.38±24.26
Significance		[1]P= 0.320	[1]P=0.0001 [2]P=0.0001	[1]P=0.622 [2]P= 0.123	[1]P=0.0001 [2]P= 0.0001	[1]P=0.016 [2]P= 0.096
Liver weight (gram)	8.22±0.59	6.66±0.50	10.39±0.90	7.92±1.01	8.23±0.79	8.46±0.98
Significance		1P= 0.010	[1]P=0.001 [2]P= 0.0001	[1]P=0.591 [2]P= 0.024	[1]P=0.986 [2]P= 0.010	[1]P=0.679 [2]P= 0.004
Spleen weight (gram)	0.80±0.14	0.65±0.20	0.85±0.05	0.61±0.12	0.76±0.08	0.62±0.19
Significance		[1]P= 0.134	[1]P=0.680 [2]P= 0.059	[1]P=0.059 [2]P= 0.649	[1]P=0.698 [2]P= 0.265	[1]P=0.091 [2]P= 0.762
Right kidney weight (gram)	0.98±0.17	1.02±0.18	1.04±0.07	0.96±0.08	1.22±0.09	1.05±0.04
Significance		[1]P= 0.618	[1]P=0.455 [2]P= 0.770	[1]P=0.874 [2]P= 0.487	[1]P=0.010 [2]P= 0.022	[1]P=0.421 [2]P= 0.724
Left kidney weight (gram)	0.81±0.15	1.02±0.15	1.03±0.09	0.99±0.04	1.21±0.07	1.02±0.01
Significance		[1]P= 0.007	[1]P=0.007 [2]P= 0.920	[1]P=0.014 [2]P= 0.714	[1]P=0.0001 [2]P= 0.010	[1]P=0.008 [2]P= 0.948
Heart weight (gram)	0.83±0.13	0.74±0.09	0.97±0.06	0.86±0.13	0.88±0.06	0.79±0.10
Significance		[1]P= 0.189	[1]P=0.058 [2]P= 0.002	[1]P=0.655 [2]P= 0.069	[1]P=0.489 [2]P= 0.048	[1]P=0.555 [2]P= 0.475
Right testis weight (gram)	1.50±0.09	1.27±0.20	1.56±0.02	1.23±0.11	1.45±0.04	1.11±0.51
Significance		[1]P= 0.155	[1]P=0.702 [2]P= 0.074	[1]P=0.090 [2]P= 0.753	[1]P=0.759 [2]P= 0.264	[1]P=0.025 [2]P= 0.294
Left testis weight (gram)	1.50±0.10	1.26±0.26	1.57±0.05	1.25±0.11	1.58±0.10	1.07±0.52
Significance		[1]P= 0.157	[1]P=0.691 [2]P= 0.073	[1]P=0.151 [2]P= 0.980	[1]P=0.640 [2]P= 0.063	[1]P=0.021 [2]P= 0.255

Data are expressed as mean+/- Standard deviation
[1]P: Significance versus control; [2]P: Significance versus gentamicin using One-Way ANOVA test (LSD).

Table 2. Comparison of changes in percentage of organs weights in different studied groups.

Parameters	Control untreated	Gentamicin treated	Fenugreek	Gentamicin + Fenugreek	Gentamicin + Nifedipine	Gentamicin + Fenugreek + Nifedipine
Liver weight (%)	3.69±0.42	3.09±0.19	3.50±0.29	3.49±0.48	3.00±0.29	4.28±0.93
Significance		[1]P= 0.079	[1]P=0.577 [2]P= 0.224	[1]P=0.537 [2]P= 0.209	[1]P=0.056 [2]P= 0.776	[1]P=0.102 [2]P= 0.002
Spleen weight (%)	0.36±0.06	0.30±0.09	0.28±0.02	0.27±0.05	0.28±0.03	0.32±0.13
Significance		[1]P= 0.258	[1]P=0.167 [2]P= 0.731	[1]P=0.082 [2]P= 0.487	[1]P=0.133 [2]P= 0.630	[1]P=0.458 [2]P= 0.718
Right kidney weight (%)	0.44±0.08	0.47±0.10	0.35±0.02	0.42±0.04	0.44±0.03	0.53±0.08
Significance		[1]P= 0.422	[1]P=0.077 [2]P= 0.011	[1]P=0.766 [2]P= 0.248	[1]P=0.896 [2]P= 0.504	[1]P=0.070 [2]P= 0.244
Left kidney weight (%)	0.36±0.08	0.47±0.07	0.35±0.03	0.44±0.04	0.44±0.02	0.51±0.06
Significance		[1]P= 0.008	[1]P=0.616 [2]P= 0.002	[1]P=0.058 [2]P= 0.321	[1]P=0.060 [2]P= 0.395	[1]P=0.001 [2]P= 0.289
Heart weight (%)	0.37±0.04	0.34±0.04	0.33±0.02	0.38±0.06	0.32±0.03	0.39±0.05
Significance		[1]P= 0.336	[1]P=0.168 [2]P= 0.608	[1]P=0.757 [2]P= 0.183	[1]P=0.111 [2]P= 0.450	[1]P=0.446 [2]P= 0.086
Right testis weight (%)	0.67±0.01	0.59±0.10	0.53±0.01	0.54±0.06	0.53±0.01	0.56±0.28
Significance		[1]P= 0.338	[1]P=0.101 [2]P= 0.414	[1]P=0.119 [2]P= 0.499	[1]P=0.104 [2]P= 0.426	[1]P=0.187 [2]P= 0.651
Left testis weight (%)	0.67±0.01	0.59±0.13	0.53±0.02	0.55±0.06	0.58±0.03	0.53±0.28
Significance		[1]P= 0.329	[1]P=0.128 [2]P= 0.509	[1]P=0.180 [2]P= 0.683	[1]P=0.302 [2]P= 0.909	[1]P=0.142 [2]P= 0.547

Percentage of organ weight was calculated as organ weight divided by total body weight multiplied by 100. Data are expressed as mean+/- Standard deviation
[1]P: Significance versus control; [2]P: Significance versus gentamicin using One-Way ANOVA test (LSD).

Table 3. Comparisons of kidney and liver function tests and lipid profile in different studied groups.

Parameters	Control untreated	Gentamicin treated	Fenugreek	Gentamicin + Fenugreek	Gentamicin + Nifedipine	Gentamicin + Fenugreek + Nifedipine
Creatinine (umol/L)	42.75±8.06	98.20±48.33	43.50±4.04	102.00±42.94	92.00±2.45	106.00±37.85
Significance		^{1}P= 0.020	^{1}P=0.974 ^{2}P= 0.021	^{1}P=0.014 ^{2}P= 0.856	^{1}P=0.045 ^{2}P= 0.780	^{1}P=0.013 ^{2}P= 0.725
Albumin (g/L)	11.50±1.29	10.40±1.67	11.50±1.73	10.80±1.10	11.00±0.82	10.98±0.82
Significance		^{1}P= 0.223	^{1}P=1.000 ^{2}P= 0.223	^{1}P=0.433 ^{2}P= 0.633	^{1}P=0.594, ^{2}P= 0.501	^{1}P=0.575, ^{2}P= 0.518
Blood urea nitrogen (mmol/L)	9.00±3.32	20.26±13.17	7.95±1.21	20.90±14.16	15.70±3.02	19.20±5.12
Significance		^{1}P= 0.079	^{1}P=0.871 ^{2}P= 0.056	^{1}P=0.064 ^{2}P= 0.912	^{1}P=0.308 ^{2}P= 0.461	^{1}P=0.127 ^{2}P= 0.863
Uric acid (umol/L)	94.50±20.44	122.80±38.67	97.00±36.95	71.20±16.21	72.50±7.76	78.25±17.91
Significance		^{1}P= 0.120	^{1}P=0.893 ^{2}P= 0.155	^{1}P=0.197 ^{2}P= 0.005	^{1}P=0.246, ^{2}P= 0.009	^{1}P=0.387, ^{2}P= 0.019
Cholesterol (mmol/L)	1.40±0.42	1.89±0.41	1.56±0.09	2.21±0.51	2.74±0.18	1.89±0.51
Significance		^{1}P= 0.077	^{1}P=0.568 ^{2}P= 0.225	^{1}P=0.006 ^{2}P= 0.218	^{1}P=0.0001 ^{2}P= 0.005	^{1}P=0.095 ^{2}P= 0.987
Triglyceride (mmol/L)	0.35±0.13	0.67±0.18	0.49±0.21	0.90±0.16	0.500.08	0.54±0.10
Significance		^{1}P= 0.005	^{1}P=0.203 ^{2}P= 0.093	^{1}P=0.0001 ^{2}P= 0.025	^{1}P=0.181 ^{2}P= 0.107	^{1}P=0.089 ^{2}P= 0.219
Low density lipoprotein cholesterol (mmol/L)	0.29±0.13	0.31±0.07	0.33±0.01	0.41±0.09	0.56±0.05	0.35±0.11
Significance		^{1}P= 0.673	^{1}P=0.541 ^{2}P= 0.822	^{1}P=0.039 ^{2}P= 0.073	^{1}P=0.0001 ^{2}P= 0.0001	^{1}P=0.294 ^{2}P= 0.487
High density lipoprotein cholesterol (mmol/L)	1.26±0.36	1.72±0.40	1.33±0.09	1.82±0.56	2.44±0.09	1.69±0.38
Significance		^{1}P= 0.078	^{1}P=0.800 ^{2}P= 0.129	^{1}P=0.035 ^{2}P= 0.675	^{1}P=0.0001 ^{2}P= 0.009	^{1}P=0.117 ^{2}P= 0.898

Data are expressed as mean+/- Standard deviation
^{1}P: Significance versus control; ^{2}P: Significance versus gentamicin using One-Way ANOVA test (LSD).

Histology of kidney in gentamicin + nifedipine drug administrated group

In this group, it was observed that nifedipine provides protection against gentamicin-induced histological features of nephrotoxicity, especially changes in glomerular tufts (Figures 2, 3). Electron microscopic evaluation revealed proximal epithelial cells having normal brush border with mitochondrial population and a well-defined nucleus (Figure 5).

Histology of kidney in gentamicin +fenugreek seeds + nifedipine drug administrated group

Mild or no protection was offered by combination of fenugreek seeds and nifedipine where kidney parenchyma showed damaging effect of renal tubules and glomeruli (Figures 2, 4). Electron microscopic evaluations showed intact cells with normal nuclei and healthy brush border. The cells contained lipid droplets and few deformed mitochondria (Figure 5).

Discussion

Gentamicin nephrotoxicity limits its usage against Gram-negative bacteria. It is also used as a tool to induce acute nephrotoxicity in experimental animals. In most investigations, oxidative stress plays a central role in induction of nephrotoxicity by gentamicin. Antioxidants have been found to be protective agents against gentamicin nephrotoxicity [14-17]. Fenugreek seeds are potential antioxidants due to their phytochemical constituents like polyphenolic flavonoids, steroid saponins/ steroidal sapogenins, polysaccharides mostly galactomannans and 4-hydroxy isoleucine [18]. On the other hand, nifedipine is another potential nephroprotective agent as it significantly attenuates the changes in tissue and urine total nitric oxide levels, and renal oxidative stress thereby prevents morphological changes in kidney [11, 19, 20].

In the present study, we investigated the effect of fenugreek seed extract and nifedipine during gentamicin-induced acute renal failure in rats. We observed a two-fold increase in blood creatinine level, which indicates distinct renal structural damage. An increase in urea and creatinine serum levels was observed due to gentamicin toxicity, as these parameters essentially depend on the glomerular filtration, which in turn is impacted by gentamicin treatment. Nifedipine and fenugreek treatment showed marked alleviation in the gentamicin-induced changes in serum creatinine and uric acid levels. As reported in previous studies, gentamicin-induced weight loss results from renal tubular injury and increased catabolism resulting in acidosis, impaired dehydration, and anorexia [14, 21, 22]. But, in the present study weight loss caused by gentamicin was ablated to a great extent by fenugreek seed extract treatment (Table 1) In the present work, we observed severe and extensive histological alterations in the renal tissue caused by gentamicin mediated oxidative damage to macromolecules and cellular organelles, which is in concurrence with previous studies [7, 14, 23-25].

The histopathology results demonstrated the cellular anti-inflammatory property of fenugreek seed extract as its treatment to the group showed ablated inflammatory events, most likely due to polyphenols, especially flavonoids, and/or 4-hydroxy isoleucine present in fenugreek seed extract.

Balakumar et al. reported that gentamicin-induced acute renal failure occurs due to necrosis, mainly in proximal convoluted tubules, which is characterized by cellular desquamation, parenchymal degeneration and tubular obstruction causing marked nephrotoxicity [21]. The tubular necrosis and degeneration observed in the present work conform to the findings of earlier investigations [24, 26].

Chade et al. reported that increased susceptibility to end-stage renal disease, development, and progression of atherosclerotic process and fibrosis in the stenotic kidney is caused by hypercholesterolemia [27]. Our results showed that the treatment with gentamicin + nifedipine significantly elevated serum TC, LDL, and HDL levels when compared to both the control group and gentamicin treated group. Rashid et al. observed that gentamicin treated rats showed increased serum cholesterol and also caused prominent damage in the kidney tissue [28]. In our study, treatment with gentamicin + fenugreek combination showed a significant increase in triglyceride levels, which in itself is an important risk factor because it influences lipid deposition and clotting mechanism [29].

Hypercholesterolemia, as observed in the Gentamicin + Nifedipine group as compared to the Gentamicin + Fenugreek + Nifedipine group, may be attributed to the presence of polyphenols, especially flavonoids, and/or 4-hydroxy isoleucine in fenugreek seed extract [30-34].

The nephroprotective potential of nifedipine and fenugreek seed extract can be a result of the increase in the activities of antioxidant enzymes in the proximal tubule that in turn reduces the production of gentamicin-induced reactive oxygen species, thereby also halts progress of apoptosis [30, 31, 35].

Conclusion: Administration of fenugreek seed water extract was found to provide potential or mild protection against gentamicin-induced nephrotoxicity in rats. The results of the study have demonstrated the protective role of fenugreek and nifedipine against the extensive damage caused by the gentamicin treatment.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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